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Observations on Aujeszky's Disease in Northern Ireland

by

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BELFAST

PART I

INTRODUCTION

In 1938 Harvey and Reid described symptoms in two bovines typical of those seen in cases of Aujeszky's disease. These two cases occurred in Cornwall. Attempts to transmit the disease failed.

The publication of this article was followed by descriptions of cases seen by Hart (1938) in County Fermanagh, Northern Ireland, in 1912, 1914, 1921 and again in 1934. McCorry (1938) described two cases seen by him in 1914, also in County Fermanagh. The cases seen by Hart and McCorry all occurred in milch cows. Stewart (1938) described a case which he saw in a Cheviot ewe in County Down in 1936. In October, 1938, the writer heard of two cases in sheep at Ballycastle, Co. Antrim, and at once visited the farm. Unfortunately the carcases had been destroyed, but the symptoms described by the farmer were typical of Aujeszky's disease.

In March, 1939, the writer was consulted by Mr. D. F. McKelvey, M.R.C.V.S., D.V.S.M., Lisburn, about three peculiar cases in bovines. These cases were investigated and the virus of Aujeszky's disease recovered from them. This is the first record of the confirmation of the disease in the British Isles (Lamont & Kerr, 1939). Other cases have been confirmed in pigs, cats and dogs by Lamont & Shanks (1939), Lamont (1940), Cassells & Lamont (1942).

In all, the writer has obtained records of 23 outbreaks of this disease in Northern Ireland involving 56 animals, *viz.*, 21 bovines, three sheep, 28 pigs, three dogs and one cat. The virus has been recovered in eight of these outbreaks. In the other cases this was not attempted for various reasons. In two cases showing typical symptoms the author failed to recover the virus. In this connection, however, it is worth noting that various portions of the central nervous system should be used for animal inoculations and particularly portions of the spinal cord near the abraded area before negativing the presence of Aujeszky's disease.

TRANSMISSION EXPERIMENTS

Successful attempts to transmit the disease to calves, pigs, guinea-pigs, rabbits and rats were made. The material used was prepared by taking small portions from various parts of the central nervous system and rubbing them up in a mortar with Hartley's digest broth until a "smooth" cream was obtained. The M.L.D. of this cream or suspension was estimated on guinea-pigs. Unless where otherwise stated the bovine strain of virus first isolated by Lamont & Kerr (1938) was used.

Bovine.

Two four-month-old calves were inoculated subcutaneously in front of the brisket with ten guinea-pig M.L.D.s. One calf developed symptoms on the fourth day following inoculation, the other calf developed symptoms on the fifth day. Symptoms comprised intense pruritus at the site of inoculation and also at the inner side of the foreleg on the side of inoculation and over the mane region. Diaphragmatic spasm was very pronounced. Temperatures of 104° and 103·8° F. were recorded in both calves respectively. In each case death occurred in less than 24 hours from the onset of symptoms.

Post-mortem Findings.—In addition to the abraded areas resulting from the scratching and rubbing, areas of congestion were present in the lungs and petechiae and ecchymoses were present in the myocardium. The liver was a light yellowish colour. The meninges of the brain were very congested. Virus was recovered from the brains of both calves.

In ingestion experiments four calves were fed with approximately 1,000 guinea-pig M.L.D. with negative results. Virus neutralizing antibodies did not develop in their blood sera.

Scarification Experiments.—A calf between three and four weeks old was used for this experiment. A small area of skin about 6 or 8 cm. below the anus was shaved closely and into the reddened area 2 c.c. of a 20 per cent. bovine brain suspension was rubbed with a small piece of wood, the idea being to simulate as far as possible the natural tendency of animals to rub this area which many animals indulge in against "rubbing posts," etc. Two days later the calf was nibbling and attempting to bite this area. As the symptoms progressed rapidly it was destroyed on humanitarian grounds.

On *post-mortem* examination a deep purple-red weal was noted around the lower margin of the abraded site of scarification. Dark, deeply congested areas were present in the lungs involving principally the anterior, the cardiac and frontal portions of the main diaphragmatic lobes.

Intranasal Route.—Two calves were used in this experiment. The inoculum used was a 20 per cent. suspension of the stock rabbit brain (M.L.D. for guinea-pig 5 mg.). The calf's nose was elevated and the material injected into the nostril. No. 1 calf, which was one week old, received 10 c.c. The result was negative. No. 2 calf received 3 c.c. into the right nostril. This calf sneezed and no further material was injected. On the third day the calf was noticed rubbing its right nostril against the wall. The pruritus became more intense and the calf died within ten hours from the onset of the symptoms.

On *post-mortem* examination dark, almost black, glistening areas were present in the frontal lobes of both lungs.

Equine

All animals used were tested before inoculation for the presence of virus neutralizing bodies in the blood stream with negative results. They were all aged horses and placed at the author's disposal by a local knackery.

The material used was a suspension of rabbit brains. Five animals were inoculated subcutaneously with negative results. The dose varied from 100 guinea-pig M.L.D.s to 250 guinea-pig M.L.D.s. Two ingestion experiments using 400 guinea-pig M.L.D.s in each case also gave negative results.

In two other experiments there was some reason to believe that a positive transmission had occurred. The first animal received a series of injections. The initial injections were made at two sites over the facial nerve and over the posterior thoracic nerves. One hundred guinea-pig M.L.D.s were inoculated at each site. No symptoms were observed and nine days later 550 guinea-pig M.L.D.s were injected subcutaneously. Eight days later a rabbit brain suspension containing 1,800 guinea-pig M.L.D.s was mixed with a mash and fed to the animal—an aged blood mare. No symptoms were observed and five days later a further dose of 800 guinea-pig M.L.D.s was injected subcutaneously; on the following day, *i.e.*, on the 23rd day from the first injection, the attendant reported that the mare was dull and sleepy. The mare was found standing with her head in the corner of the loose box; she was dull and had a drowsy, somnolent expression. She was also rather stiff as if suffering from rheumatism. The temperature was normal. On the 30th day a mucopurulent conjunctivitis developed but cleared up spontaneously within a few days. On the 45th day the mare appeared quite normal. A blood-sample was collected and found to contain virus neutralizing antibodies.

In the second case the animal was an aged black gelding. The infecting dose was prepared from rabbit brain the M.L.D. of which was 5 mg. The quantity of brain used was 7·1 grammes suspended in Hartley's digest broth. Two grammes were injected deeply into the left breast and 5·1 grammes were injected over the site of the left posteror thoracic nerves. The horse was examined daily and the temperature recorded.

On the 13th day following inoculation the horse was found to be very stiff and sleepy. He swayed on his hind legs and gave the impression that he would collapse. His gait was uncertain and stumbling and his powers of balancing were affected. In the loose box he stood with his hindquarters propped against the wall. The under lip and penis were pendulous. The pulse was soft and difficult to detect, rate 65. The conjunctival mucous membrane was a dirty yellowish colour. Temperature 100·5° F. The horse fed and drank slowly. Some pruritus was present at the site of inoculation over the posterior thoracic nerves, but this lasted only a few hours and did not recur. Trembling of the hindquarters was noted.

On the 14th day the symptoms were more pronounced and it appeared as if the horse would fall. Stiffness was very pronounced and walking a matter of great difficulty. A blood sample was drawn from the jugular into citrate and 5 c.c. inoculated into a rabbit subcutaneously. No symptoms developed in the rabbit.

On the 15th day the horse was slightly better and steady improvement took place each day thereafter. On the 22nd day the horse was practically normal. On the 27th day the horse was found blind on examination although no abnormality was detected in his eyes.

On the 30th day following the initial inoculation an attack of conjunctivitis developed. A "thin" mucopurulent discharge was present. This cleared up in a few days. During the course of the illness the horse became noticeably thinner.

A blood sample was tested for the presence of virus neutralizing antibodies on the 25th day with positive results.

These two cases are presented in some detail as the symptoms are not dissimilar to those described by other workers. Remlinger & Bailly (1938) describe the following symptoms in the equine: "loss of appetite, depression and prostration; attacks of terror without apparent cause have been noted. The condition gets worse fairly quickly. The eyes become very sunken, the walk is drunken because of the trembling of the hindquarters. If the animal is forced to walk it scrapes the ground, stumbles at the slightest unevenness and sometimes collapses heavily. Itching does not occur in all cases and it does not show anything like the dramatic appearance seen in dogs, cats and cattle. The signs of paralysis soon take the upper hand. Along with these are usually signs of agitation and even of madness. Frequently the horse pushes against the wall with all its strength, supporting itself with the head or neck. Sometimes it bites at the feeding trough and partitions. Most reflexes disappear. Incontinence, paralysis of the penis, grinding of the teeth and salivation have all been observed. The animal falls down as paralysis progresses. Sometimes it struggles for a long time before dying.

"The disease may last 24 hours but with horses the development may take much longer; two, three and even four days. The prognosis is very grave but not fatal. Cases of recovery have been seen. Lourens (1935) reported three cases of natural disease and two of artificial infection which recovered spontaneously."

Braga & Faria (1934) inoculated six horses and three mules without producing any signs of the disease in spite of large doses of virus injected subcutaneously, intramuscularly and even below the dura mater. Similar difficulty in infecting the equine was experienced by Gerlach & Schweinburg (1935). These workers failed to infect two horses and one donkey by subcutaneous injection of a whole rabbit's brain to each animal. They state their virus was highly virulent for rabbits and was freshly passaged. A third horse, a five-year-old gelding, became ill 19 days after intramuscular injection with 10 c.c. of "a very thick emulsion prepared from seven different strains of the virus, *i.e.*, after an extraordinarily long incubation period for this disease." This horse was injected intramuscularly in the left shoulder and remained well until the 19th day until when, without warning, it showed "a completely changed behaviour, restlessness, tendency to be frightened, occasional severe shaking of the hindquarters when at rest and kicking with the hind feet at the walls without any reason." On the next day these symptoms were more pronounced. The animal "looked stupid and was very depressed." There was constant scraping with the left foreleg. It crouched in the corner and pressed its nose against the walls and supported its head on the feeding trough. With the forelegs wide apart it bit at the injection spot on the shoulder and at the flesh behind this spot. The horse stumbled and there was danger of it falling when turned, owing to weakness of the hindquarters. The illness was at its worst on the third and fourth days. Respiration was very difficult and the penis was protruded and pendulous. There was complete indifference to its surroundings and the horse "of its own accord put itself into unphysiological positions." The horse moved forward with considerable difficulty. There were twitchings of the head and neck and cramp of the masseters. On the fifth and sixth days the horse was slightly improved although the trembling of the hindquarters was still present. Considerable improvement was recorded on the seventh day but the horse was slaughtered as Gerlach & Schweinburg wished to subject the central nervous system to a histological examination.

During the seven days of illness the horse lost 48 kg.

Porcine

In all, 13 pigs were used for transmission experiments. The blood of all pigs was tested for virus neutralizing bodies before the experiments with negative results. Six pigs were inoculated subcutaneously, one intravenously, two intranasally, one intracranially. Three contact experiments and one ingestion experiment were also carried out. In the subcutaneous route the dose varied from 8 G.P. M.L.D.s up to 400 G.P. M.L.D.s. No obvious symptoms of ill health were shown by these pigs but virus neutralizing bodies appeared in their blood stream. The pigs used were from five to six weeks old.

Two pigs aged six weeks were inoculated intranasally with 400 and 200 G.P. M.L.D.s respectively. No symptoms were noted but virus neutralizing bodies appeared in the blood streams.

In the in-contact experiments three eight-weeks-old pigs were housed with a pig from which a potent antiserum was later obtained. This pig received a series of injections of virus. No symptoms of illness appeared in the in-contacts. The duration of contact was three months. No antibody formation was detected in their blood streams at the end of three months.

In the second in-contact experiment four pigs were housed with one of the pigs which was injected intranasally. Before the experiment the blood sera of all five pigs were tested for virus neutralizing antibodies with negative results. At the end of 14 days the blood sera were again tested. Antibody formation was found in the pig which was inoculated intranasally but not in the four in-contact pigs:—

A rat which had died from Aujeszky's disease was placed in a pen with three pigs. The pigs were observed to move the rat with their noses and one of them picked the rat up in its mouth but dropped it again. The rat was removed from the pen after one hour. No symptoms of illness developed in these pigs and no antibody formation was detected in their blood streams on test one month later.

A six-weeks-old pig was anaesthetized with nembutal. Two G.P. M.L.D.s were injected intracranially. The pig's temperature was 103° F. On the following day the temperature was 103·2° F. and the pig was nervous and excitable. On the second day the pig was dull and recumbent. The temperature was 106·2° F. On the third day the pig was showing symptoms of facial paralysis and walking in circles. Its temperature was 108·2° F. During this examination a fit developed, the pig collapsed and died almost immediately. Virus was recovered from its blood stream.

A pig inoculated intravenously with 5 c.c. filtrate prepared by filtering through a coarse filter a 10 per cent. emulsion of rabbit brain, showed shivering fits on the second day and the temperature rose from 103·2° F. to 105·4° F. These symptoms persisted for three days. The pig recovered. Its blood serum was tested one week later for antibody formation with positive results.

Discussion

In the naturally occurring outbreak described by Lamont & Shanks (1939) two litters of young pigs succumbed after showing symptoms of nervous derangement. An older litter in contact remained healthy. Other writers, *e.g.*, Hirt (1935), Shope (1934, 1935), Koves & Hirt (1934), Steiner & Lopez (1935), and Pannisset (1935) emphasize the serious nature of the disease in very young pigs. Older pigs, however, may become infected, develop antibody formation in their blood stream and show no symptoms of ill health. The experiments described suggest an age resistance.

The writer was unable to infect poultry. Remlinger & Bailly (1938), however, were able to infect poultry by the intracerebral route.

The Antigenic Relationship of Various Strains of Virus Isolated

The following strains of virus isolated in Northern Ireland were available for comparison: three bovine strains, two pig strains, one cat strain and one canine strain. A Hungarian strain was made available to the author by courtesy of Mr. R. E. Glover of the Medical Research Council. It was found that all these strains were neutralized effectively by an antiserum prepared against the first bovine strain isolated by the author.

It was noted that after several passages through rabbits all these strains behaved in an identical manner in regard to period of incubation and symptoms produced in rabbits and guinea-pigs. The author was unable to show any relationship between the virus of piglet influenza (1938) and the virus of Aujeszky disease by cross immunity tests. Shope (1935) showed that the virus of swine influenza and the virus of Aujeszky disease were dissimilar.

Longevity of Virus

Various authors have commented on the longevity of the virus when preserved in 50 per cent. glycerine and maintained at temperatures of from 0° C. to + 6° C. (*e.g.*, Remlinger & Bailly (1935), Lourens (1935), Galloway (1938), etc.

The author's experience indicates that the virus does not remain active indefinitely: virus containing brain preserved in glycerine was found to be virulent for 18 months but not for two years.

In an attempt to investigate this point, pooled rabbit brains of a known G.P. M.L.D. were preserved in 50 per cent. glycerine in the cold room. No apparent diminution in virulence was noted up to the tenth month. At the end of the eleventh month it was noted that the M.L.D. for guinea-pigs had changed suddenly from x to 4x. At the end of 16 months 40x failed to set up infection.

Virus Neutralization Tests with Immune Pig Sera Prepared by Inoculating Pig with Repeated Doses of a Northern Ireland Bovine Strain (Greer) of Aujeszky Virus*

Origin of virus	Guinea-pig M.L.D.s	Result	Negative serum control
ow (Greer strain)	100	Effectively neutralized	+2
„ (Cadoo strain)	100	„	+2
„ (Law strain)...	100	„	+2
g (Harrison) ...	100	„	+2
„ (V.R.D.) ...	100	„	+2
at (Harrison) ...	100	„	+2
ungarian strain ...	100	„	+2
og (Davidson) ...	100	„	+2

+ = Death of guinea-pig in days.

* One c.c. quantities of brain suspension containing 100 guinea-g M.L.D.s was mixed with 1 c.c. of the antiserum and 1 c.c. of the egative serum control. The virus serum mixtures were allowed stand for two to two and a half hours at room temperatures and c.c. was then inoculated into guinea-pigs. A second guinea-pig as inoculated in each case with the virus antiserum mixture for nfirmatory purposes.

PART II

Location of Virus in the Body

In the rabbit and pig the author has shown by animal inoculation hat virus is present in the blood stream. The virus was recovered om the heart blood of the pig which had been infected by intra-erebral inoculation and died on the third day after inoculation.

In the rabbit, virus has been found in the blood stream at all tages of the disease from the onset of symptoms until death. his would indicate that in the rabbit the invasion of the blood eam is not a fleeting or transient phenomenon but a definite rmanent invasion.

As would be expected with a blood stream invasion, the presence f virus has been shown in a number of organs, *e.g.*, the lungs, leen, liver and kidneys. In the pig, the virus has been found in e brain and spleen, the only organs tested.

Hurst (1933), while recognizing that the virus of Aujeszky's isease has a definite predilection for the nervous system, states, s the result of histological studies in the rabbit, that the virus an parasitize cells derived from any embryonic layer in this nimal. In the pig, too, the virus exhibits multiple tissue affinities lthough in the outbreak in this species seen by the author and Ir. P. L. Shanks, nervous symptoms predominated. These sug-ested to them the possibility that they were dealing with a neuro-ropic virus.

In the rabbit the author has noted that a minute dose of virus noculated intracerebrally will produce a fatal attack, while a imilar dose of virus inoculated subcutaneously will produce no mptoms whatever. In addition it has been found that if two abbits of comparable weight from the same litter are infected ith an equal dose of virus, one intracerebrally, the other subcu-aneously, the brain of the intracerebrally infected rabbit will con-ain ten times the amount of virus when compared with that of the ubcutaneously infected rabbit (the quantity of virus present in he brains was determined by estimating the M.L.D. for guinea-igs).

The presence of virus in the blood stream in rabbits has also een studied by Remlinger & Bailly (1938). These authors have illed rabbits at intervals following inoculation of the virus into the nterior chamber of the eye. They found virus in the blood stream s early as the 25th hour following inoculation and at a time when they were unable to demonstrate the presence of virus in the ervous system. No symptoms of ill health were shown by the abbits at this stage. The nervous system remained negative for he presence of virus up to the 40th hour, although the blood was still positive. At the 46th hour the presence of virus was confirmed in the nervous tissue and it was noted that symptoms of pruritus appeared at the 48th hour in the experimental rabbits.

In the dog, rat, guinea-pig and hedgehog killed when on the oint of death, Remlinger & Bailly (1938) were unable to show that irus had invaded the blood stream. Similarly the same authors lso were unable to demonstrate the presence of virus in the blood f three young affected pigs. As has been noted already, the author has found virus present in the blood of a young pig. Remlinger & Bailly (1938) also state that they proved the blood to be virulent "once out of twice in the hen, once out of three in the cat." In a single experiment at Tangiers the virus was found in the blood of a spaniel experimentally infected (cited by Remlinger & Bailly (1938)). The author was fortunate enough to see a naturally occurring case in a cow. The disease was far advanced, the animal being recumbent and becoming very feeble. The animal had shown symptoms for almost 24 hours. A blood sample was collected from the jugular vein and citrated; 8 c.c. of this sample was inoculated into a young susceptible rabbit with completely negative results. There can be no question of the sodium citrate affecting the viability of the virus, as the author has regularly drawn off blood samples from rabbits and preserved them under exactly similar circumstances and found no difficulty in demonstrating the presence of virus with much smaller quantities of blood. In this cow the medulla and cord were found to contain the virus, although no virus was found in an equivalent weight of the cerebral hemispheres prepared and inoculated under identical conditions. This cow showed symptoms of pruritus in the vulvar and anal region and the author's experience of experimental inoculation of the virus indicates that there is a direct connection between the pruritic area and the entry site of the virus. The suggestion is put forward that in this cow the virus was travelling to the central nervous system *via* the nerve paths. The cow was destroyed and it is not unlikely that if the disease had been allowed to run its course the virus would have been recovered from the cerebral hemispheres. In four other cases occurring in bovines where death resulted from the disease the author was able with identical technique to demonstrate the presence of virus in the cerebral hemispheres.

The following experiments negative the possibility of a blood invasion in the bovine. Three c.c. of blood aspirated from the heart of a calf which had died from Aujeszky's disease following intranasal infection was injected subcutaneously into a rabbit with negative results. From another calf infected by scarification of the fine skin between the thighs, a blood sample was collected, about six hours after the onset of symptoms, when the disease appeared to be at its peak. Five c.c. of this blood was at once inoculated into a susceptible rabbit with negative results. This calf was destroyed three hours later when *in extremis* and although the presence of virus was shown in the lumbar region of the spinal cord, further examination of the blood gave negative results.

Therefore, in three bovine cases investigated no virus was found in the blood stream.

In the calf which died from intranasal inoculation virus was found in the brain, the olfactory bulbs and also in pneumonic areas in the lungs. In the calf killed after infection by scarification virus was found in the lumbar region of the spinal cord but not in the cervical region or in the brain. This finding would tend to confirm that in the bovine, virus invades the body *via* the nerve paths.

Remlinger & Bailly (1938) take the view that a blood invasion occurs and explain their failures to demonstrate the virus by the suggestion that "it is probable that the phase in the blood is early, of short duration and difficult to catch."

The method by which the virus spreads from the site of inoculation to the nervous system has occasioned some discussion. The fact that virus can be found in the blood stream of the rabbit before symptoms are shown has suggested to Schmiedhoffer (1910), Remlinger & Bailly (1933) and Manninger & Marcis (1937) that the virus reaches the central nervous system *via* the blood stream and not along the nerve paths. Hurst (1934) states that "after intravenous inoculation effective with even small doses, virus is rapidly removed from the blood and multiple infective foci are established in various organs, thence ascent of the virus by the peripheral nerves leads to infection of the central nervous system, the symptomatology differing according to whether the spinal cord or the medulla is first reached. The lack of evidence that the virus can penetrate directly the haemato-encephalic barrier deserves emphasis." The strain of virus used by the author does not disappear from the blood stream of rabbits so that apparently the Iowa strain used by Hurst behaved differently. Hurst (1934) however mentions that the Iowa strain after repeated passage was invading the blood stream. After intravenous inoculation of massive doses of virus Hurst found that at the end of 30 minutes a small quantity of virus is still present in the blood but after two hours all or almost all has disappeared. Although virus could not be found in the blood stream Hurst found virus in the organs, hence his statement regarding infective foci quoted above. He has also confirmed this point histologically.

In this connection the author carried out the following experiment. A 20 per cent. suspension of rabbit brain was prepared in Hartley's digest broth (M.L.D. for guinea-pigs 1 c.c. of a 0·5 per cent. suspension). The suspension was lightly centrifuged for a few minutes and 1 c.c. of the supernatant fluid was in-

oculated intravenously into the marginal vein of the right ear of a rabbit. After the lapse of three hours 5 c.c. of blood was drawn off into citrate from the marginal vein of the left ear and inoculated into a rabbit subcutaneously. After the lapse of ten hours the rabbit was anaesthetized with ether and immediately death ensued, the thoracic cavity was opened and 5 c.c. of blood withdrawn from the still functioning heart. The liver, spleen and kidney were then emulsified (using ground glass in a mortar) and extracted with Hartley's digest broth. The extracts were then inoculated in 5 c.c. quantities into rabbits. A 40 per cent. brain emulsion was also prepared and 3 c.c. of this was inoculated into another rabbit. In view of Hurst's (1934) findings the results were surprising; virus was present in the three-hour sample of blood, in the ten-hour sample of blood and, as might be expected, in the liver, spleen and kidney. A very interesting point, however, is that the presence of virus in the brain of the rabbit could not be proved although virus was present in the blood stream.

Koves (1935) states that in many cases he has succeeded in infecting dogs and cats by inoculating virus into the lower parts of the limbs, after resection of the corresponding nerve supply, without a modification of the period of incubation.

The following experiments in this connection were carried out by the author on rabbits.

A rabbit was anaesthetized with ether. After removal of the wool and cleaning and disinfection of the skin over the thigh muscles a longitudinal incision was made through the skin, roughly parallel to the femur and about 1·25 cm. posterior to it. The muscle mass was carefully divided in the same direction as the muscle bundles and the sciatic nerve exposed for approximately 5 cm. of its length. A small spatula was inserted below the nerve to raise it and a fine bore needle inserted beneath the nerve sheath; a minute drop of a 20 per cent. rabbit brain suspension (M.L.D. for guinea-pigs 1 c.c. of 0·5 per cent. suspension) was injected into the nerve. As the needle was withdrawn it was noticed that a slight leak of virus occurred. The nerve was sectioned close to the spinal canal 2·6 cm. above the site of inoculation of the virus and 0·2 cm. of its length removed. The wound was closed with interrupted sutures. The rabbit died on the third day from a typical attack of Aujeszky's disease. The rabbit manifested the usual symptoms of pruritus but the interesting point was that the pruritic area was distal to the site of inoculation in the tendo-Achilles region. After death the presence of virus in the lumbar region of the cord was proved by inoculation of a rabbit. No pruritus occurred at the site of operation and the divided muscle was healing normally. The inoculated nerve was surrounded by a small area of haemorrhagic oedema. The nerve was soft and oedematous. The uninoculated portion of sciatic nerve entering the cord was firm and appeared quite normal.

This experiment was repeated on a second rabbit. The needle before insertion beneath the nerve sheath was freed from all traces of virus; 0·01 c.c. of a 20 per cent. suspension of rabbit brain was injected into the nerve. This minute drop caused slight tensing of the nerve sheath. The needle was then withdrawn slightly in the nerve sheath and the piston of the syringe slightly eased. The needle was then withdrawn. No leakage occurred. The nerve was divided as described above and the divided inoculated portion trapped in the muscle bundles and the wound closed. This rabbit showed typical symptoms of the disease early on the fourth morning following inoculation. It died on the fourth day. It was noted that the pruritic area was in the tendo-Achilles region quite clear of the operation site. A rabbit of comparable weight inoculated with a similar dose subcutaneously in the hind leg also died on the fourth day some five hours earlier.

A *post-mortem* examination of the site of operation was made. It was noted that the skin and muscle wound had commenced to heal. The divided ends of the nerve were exposed and it was found that they were separated by a distance of 1·2 cm. with some muscle tissue intervening. The inoculated portion of the nerve was surrounded by a haemorrhagic oedematous zone and had lost its firmness and appeared soft and pulpy. The proximal part of the nerve was pearly white in appearance and quite firm by contrast. The spinal cord immediately in front of the sciatic branching was tested for presence of virus by rabbit inoculation with positive results.

These two experiments indicate that section of the main nerve supply to the hind limb has no effect whatever on the invasion of the body by the virus of Aujeszky's disease. Symptoms and death followed in the normal time. Introduction of the virus into the distal portion of the divided nerve also in no way affected the period of incubation. It seems clear that the strain of virus with which the author has been experimenting is very well adapted to the rabbit. It is not unreasonable to assume that the virus had multiplied quickly at the site of inoculation and had either reached the sectioned end of the sciatic nerve by diffusion through the tissues or had rapidly invaded the blood stream and reached the end of the sciatic nerve in this manner.

The manifestation of pruritus towards the terminal distribution of the sciatic nerve requires some consideration. Hurst (1933) is of the opinion that the symptoms of pruritus are not due to irritation of nerve endings but to inflammation of the corresponding spinal ganglion. In support of this view he states that "if inoculation is practised in the calf muscles, provided that care be taken to prevent leakage into the overlying skin, itching is not local but is referred to the terminal distribution of the sciatic nerve and perhaps to the posterior part of the flank of the affected side." The author has noticed this phenomenon several times following deep inoculation into the muscle tissue of the femoral region. If one assumes that section of the sciatic nerve denervates the limb below the point of division of the nerve, it is difficult to explain the undeniable symptoms of pruritus, present in both rabbits, on any other basis than inflammation of the corresponding spinal ganglion. Hurst (1933) in support of this view has noted that about 50 per cent. of rabbits inoculated intravenously itch at some point (unrelated to the site of venepuncture) and lesions are then present in the corresponding spinal ganglion. As already mentioned, Hurst explains this phenomenon on the grounds that the virus becomes localized in certain organs and the virus reaches the nervous system *via* the nerve supply to these organs. Hurst has also inoculated virus directly into certain internal organs such as the kidneys, liver and lungs. Itching has resulted on the flank and on the shoulder thus giving additional confirmation to this view.

During the course of this work the author has noted the following points:—

1. On two occasions rabbits were killed at the first onset of the pruritic symptoms and suspensions of the cerebral hemispheres prepared. These were tested for virus content by animal inoculation with negative results. In both these rabbits the infective dose was given subcutaneously in the right hind leg and the presence of virus in the sciatic nerve and also in the lumbar portion of the cord was proved by inoculation of rabbits. In other rabbits, however, killed at the same stage, virus was found in the cerebral hemisphere.

2. Three rabbits were inoculated intracerebrally. Death occurred in 48 hours. The sciatic nerves were dissected, removed and suspended in Hartley's digest broth and injected into rabbits with positive results. Hurst (1934) notes the significant fact that if an equivalent dose of virus be inoculated at the same time into the tip of the ear, into the flank and into the hind foot, itching occurs first in the ear then in the flank and the rabbit may die before itching starts in the foot.

As the result of inoculating a considerable number of animals subcutaneously the author considers that there is a definite association between the entry site of the virus and the pruritic area. If, as stated by Hurst (1933), the pruritus is due to inflammation of the corresponding spinal ganglion one is forced to the conclusion that notwithstanding the presence of virus in the blood stream invasion takes place in the rabbit *via* the nerve tracks. The author sees no other explanation for the phenomenon noted. If the virus travelled solely by the blood stream why should it select the spinal ganglion corresponding to the site of inoculation? Again, if virus is inoculated into the sciatic nerve or deeply into the femoral muscles, pruritus occurs at the terminal distribution of the sciatic nerve. This observation strongly favours the view that the virus travels by the nerve track.

In two calves which were inoculated at the base of the neck anterior to the brisket the pruritus occurred not only at the site of inoculation but also in the mane region of the neck and the inside of the leg. Assuming that virus ascended through the neural route it would involve the spinal ganglion of the cervical nerves (sixth and seventh cervical certainly) and the brachial plexus. The dorsal branches of the cervical nerves pass upwards to the rhomboideus muscles. The itching in this site confirms Hurst's (1934) view that "itching is a referred sensation from a central lesion." Histological studies by Hurst (1933) also confirm the view that virus travels by the neural route.

The experiment in which virus was inoculated intravenously into a rabbit is also of great interest. As already described, virus was present in the blood stream and certain organs at the tenth hour but not the brain (although the infecting dose from this organ was large and representative of all parts of the brain, the author designedly avoided including blood in the inoculum as far as possible). It would appear, therefore, that although virus was present in the blood stream for as long as ten hours, no virus had penetrated the haemato-encephalic barrier.

Galloway (1938), Hurst (1934) and Remlinger & Bailly (1938) record that the virus spreads in a centrifugal manner after intracerebral inoculation. The author's experiments throw no light on this point as in three rabbits inoculated intracerebrally virus was

present in the sciatic nerves, the blood, lungs, liver and kidney in addition to the brain.

On the whole the weight of evidence favours the view that the virus invades by the neural route.

The Presence of Virus in the Blood Stream

As already mentioned, the virus of Aujeszky's disease has been found with great regularity in the blood stream of rabbits.

Remlinger & Bailly (1938) have conducted a series of experiments to determine exactly which part of the blood carries the virus. Their conclusions are that the virus is contained in the red blood corpuscles and that the white corpuscles and the serum are completely free from virus. The author has carried out some experiments in this connection.

Plasma

A rabbit which had become moribund as the result of a subcutaneous injection of Aujeszky's virus was carefully watched until death occurred. The thoracic cavity was at once opened and blood aspirated from the still functioning heart into 2·5 per cent. sodium citrate solution. In all, approximately 25 c.c. of blood was collected. The cellular elements were separated by centrifuging for 30 minutes at 1,000 revolutions per minute. The clear supernatant plasma was pipetted off and submitted to further centrifuging for three periods of 30 minutes each at 2,000 revolutions per minute. No sediment was present in the centrifuge tube at the final centrifuging and the plasma was completely clear and transparent. Five c.c. of the plasma was injected into a rabbit subcutaneously. Approximately two hours 30 minutes had elapsed from the collection of the blood until the rabbit was injected with the plasma. The rabbit developed typical pruritus on the third day following inoculation and died from a typical attack of Aujeszky's disease. The presence of virus in its brain was confirmed by inoculation of a rabbit.

Red Cells

Coincident with this experiment a second rabbit which had been injected with virus was anaesthetized with ether three hours after the onset of the pruritic symptoms. Approximately 23 c.c. of blood was aspirated from the heart into sodium citrate solution. The blood sample was centrifuged for 30 minutes and the plasma pipetted off and recentrifuged three times as already described. Five c.c. of the clear cell free plasma was injected into a rabbit subcutaneously. The rabbit developed the typical Aujeszky pruritus on the third day following inoculation. In the centrifuge tubes in which the citrated blood collected from both rabbits was centrifuged it was noticed that the white cells had formed a distinct disc or wad above the packed red cells. A wide bore capillary pipette was prepared and carefully inserted down the side of the tube to the bottom. The red cells were then slowly aspirated and discharged into clean, sterilized centrifuge tubes. It was found possible by this technique of slow aspiration from the bottom of the centrifuge tube to remove practically all the red cells and leave the layer of white cells intact. The red cells were then washed in physiological saline and recentrifuged. The technique adopted was to pipette off the supernatant fluid from the centrifuge tube, add fresh physiological saline and then bubble air through the packed red cells from a capillary pipette fitted with a rubber bulb. The red cells were brought back into suspension and thoroughly agitated by the air bubbling. Using this technique the red blood cells were washed in physiological saline eight times. A 2 c.c. volume of the packed red cells was then aspirated through a wide bore needle into a hypodermic syringe, diluted with physiological saline and injected into a rabbit. Two rabbits were inoculated with the red cells washed as described from each rabbit. On the morning of the fifth day both rabbits were affected with the typical pruritus and died in the evening. The presence of virus in their brains was confirmed by inoculation of rabbits.

White Cells

To the layer of white cells remaining in the centrifuge tubes distilled water was added and the white cells were washed in the same way as described for the red cells, substituting distilled water for the saline solution. The distilled water effectively laked the red cells. The washing was repeated on eight occasions and the white cells were completely freed from all traces of red colouring matter. The white cells from both rabbits were combined to form a composite dose which was inoculated subcutaneously into a rabbit. The volume of white cells recovered was approximately 0·4 c.c. This rabbit remained perfectly healthy, so that either no virus was adherent to the white cells or the amount of virus in the inoculum was so small that it failed to set up disease in a rabbit.

Serum

Having established that virus was present in the blood plasma of two affected rabbits, a third rabbit, which was showing symptoms, following the injection of plasma, was anaesthetized and the thoracic cavity opened; 21 c.c. of blood was aspirated from the still functioning heart. The first 10 c.c. drawn off was discharged into a sterile test tube and allowed to clot. The remaining 11 c.c. was drawn into a syringe containing citrate and after citration was discharged into a centrifuge tube. The plasma was separated as already described and the red cells were pipetted off and washed in physiological saline. The plasma and red cells were held overnight to permit the untreated blood sample to clot. The serum from the clotted sample was pipetted off and centrifuged for four further periods of 30 minutes each at 2,000 revolutions per minute. The sample of serum was clear and transparent and completely free from haemolysis. In all, 3·8 c.c. of serum was obtained from the 10 c.c. of blood. This volume was then inoculated subcutaneously into a rabbit. At the same time 3·8 c.c. of the citrated plasma was injected into a control rabbit and a third rabbit was inoculated with a 2 c.c. volume of the packed red cells.

On the third day the rabbit inoculated with plasma developed typical symptoms of Aujeszky's disease.

On the fourth day the rabbit inoculated with the serum developed symptoms of Aujeszky's disease and ten hours later the rabbit inoculated with the red cells became affected.

Remarks

In the experiments described rabbits of comparable weight and condition were used and in fact six rabbits were from the same litter. It was observed that symptoms appeared much sooner in the rabbits inoculated with plasma as compared with those inoculated with red blood cells. The author's experience in inoculating rabbits with varying doses of virus suggests that this is due to the fact that a much greater quantity of virus was present in the plasma. It should be pointed out, however, that a 5 c.c. quantity of plasma (in one experiment 3·8 c.c.) was used as compared with a 2 c.c. volume of red blood cells. If, however, the virus was specially adsorbed to or contained in the red blood cells as stated by Remlinger and Bailly and not contained in the plasma one would have expected that the rabbits inoculated with red blood cells would have been the first to show symptoms of the disease.

Summary

No further experiments with blood were carried out as these experiments show quite clearly that:—

1. Virus is commonly present in the blood of affected rabbits.
2. Virus is free in the blood plasma.
3. Virus is also free in blood serum.
4. Virus appears to have penetrated the red blood cells.

In one experiment 0·4 c.c. of white cells separated from the blood of two rabbits failed to set up disease on subcutaneous inoculation into a rabbit.

The results are at variance with those described by Remlinger and Bailly. These two workers failed to find virus in the blood plasma or serum or in the white cells, but found it present in the red blood cells.

Experiments with Blood Constituents

Material used	Result	Remarks
5 c.c. plasma ...	+3	Virus present
5 c.c. " ...	+3	"
5 c.c. " ...	+3	"
3·8 c.c. " ...	+3	"
3·8 c.c. serum ...	+4	"
2 c.c. R.B.C. ...	+5	"
2 c.c. " ...	+5	"
2 c.c. " ...	+4½	"
0·4 c.c. white cells	—	Negative result

+ = period in days elapsing from inoculation to death. Method of testing, subcutaneous inoculation into rabbits.

(*To be continued*)

Weekly Wisdom

We should go on proclaiming in season and out of season that what we mean by democracy involves toleration.—Lord Lindsay (Lords' debate on safeguards for minorities).

Artificial Insemination of Cattle in England and Wales*

An article on the practice of artificial insemination appeared in the March, 1945, issue of this journal.† At that time there were eight centres and one sub-centre in operation in this country—at Reading (operated by the National Institute for Research in Dairying and grant-aided by the Ministry), with a sub-centre at Guildford and obtaining its supplies of semen from Reading; Cambridge (also grant-aided by the Ministry); Beccles (operated by the Milk Marketing Board), and other independent centres at Avoncroft College, Bromsgrove; Dartington Hall, Totnes; Ilminster; Kimble; and Lyndhurst.

Developments since March, 1945.—The Cambridge centre was taken over by the Cambridge and District Cattle Breeders' Society on May 1st, 1945, and is now being operated by the Society as a farmers' co-operative body. On September 1st, 1945, the Milk Marketing Board acquired the Kimble centre, but later the premises there had to be given up, and, as a temporary expedient, a sub-centre, drawing supplies of semen from Reading, was opened by the Milk Marketing Board at Aylesbury. The arrangements were terminated recently when the Board opened a new main centre in temporary premises at Little Horwood, Buckingham, and Aylesbury became a sub-centre of Little Horwood. Another sub-centre of Little Horwood is now in operation at Moulton, Northants. The Board has also set up main centres at Cheswardine (Shropshire), Shincliffe (Durham) and Carmarthen, and sub-centres of Beccles and Cheswardine at Otley and Ellesmere respectively.

Since December 1st, 1945, an artifical insemination service has been provided by the Ministry's Research centre at Ruthin, which was originally established by the Ministry in November, 1943, for the express purpose of combating a wave of trichomoniasis in the Vale of Clwyd. On October 1st, 1945, the Reading centre came under the direct control of the Ministry and, whilst continuing to provide a commercial service, is also carrying out research. Sub-centres of Reading have recently been set up by the Ministry at Bloxham (Oxfordshire) and Buckland (Berkshire).

The South Lincolnshire Cattle Breeders' Society has established a centre at Kirton. Sub-centres of Lyndhurst have been opened by the Hampshire Cattle Breeders' Society at Andover and Petersfield, and Messrs. Horlicks Farms and Dairies, Ltd. are now operating sub-centres of their Ilminister centre at Castle Cary and Wells.

No bulls are kept at sub-centres: the supplies of semen required are drawn from the relevant main centres.

Developments since the publication of the last article on artificial insemination which appeared in this journal are best illustrated by a complete list of artificial insemination centres now in operation under licence granted by the Minister of Agriculture *(see on)*.

Consideration has been given to the possible interchange of supplies of semen so as to widen the operational scope of each centre. The success of such an arrangement, however, would be dependent on the practicability of transporting semen over comparatively long distances without harmful effect. This matter is still under investigation.

The National Service Progress of Work.—Progress in the provision of a national service of artificial insemination on behalf of the livestock industry must necessarily be gradual. The number of centres cannot be expected to increase rapidly in present circumstances, since shortage of building materials and labour, and other abnormal difficulties have to be faced. The following statement, however, shows that in recent months, in respect of which records have been kept of the work carried out by the various centres, progress has been encouraging, bearing in mind seasonal breeding fluctuations.

Quarter ended	Number of centres and sub-centres operating	Number of members	Number of bulls in use	Total number of cows inseminated	Estimated conception rate* *per cent.*
30.6.45	9	2,800	55	7,018	61·8
30.9.45	9	3,027	58	5,245	67·7
31.12.45	10	3,061	60	8,880	68·1
31.3.46	14	3,886	82	15,457	68·4
30.6.46	14	4,832	73	10,097	65·2

* Percentage of cows assumed to be holding to first insemination, *i.e.*, not presented for repeat insemination by the end of the following quarter.

* Reprinted from *Agriculture*, the Journal of the Ministry of Agriculture (November, 1946). 53. 364-368.

† *Agriculture*, 51. 529. Copies of a reprint of this article can be obtained, free of charge, from the Ministry of Agriculture and Fisheries (Livestock Improvement Branch), Bickenhall Mansions, London, W.1.

Artificial Insemination and Disease Control.—Artificial insemination has been shown to be of considerable assistance in preventing the spread of certain breeding diseases which are communicable by the bull—notably trichomoniasis. The operations at Ruthin in 1944 and 1945, already referred to, were highly successful in this connection. Recently, disease control centres have been opened by the Ministry in South Cardiganshire and in Caernarvonshire, with the primary objects of combating disease in those areas and of confirming the results of the experiment in the Vale of Clwyd.

Licences for Special Conditions.—In addition to centres specially set up for the purpose of artificial insemination, other bull owners have been granted licences permitting them to distribute semen for the insemination of cows registered in the same herd book as a bull with which a mating is desired, for disease treatment and research purposes.

Normally, licences of this type limit the distribution of semen to the insemination of 15 cows in not more than five herds in any period of twelve months but, in order to assist members of Cattle Breed Societies in areas where there are not enough cattle of the breed to justify the keeping of a bull of that particular breed at an artificial insemination centre, an extension beyond these limits may be approved. An arrangement of this nature has been made at the suggestion of the English Jersey Cattle Society so that owners of Jersey bulls may, under licence, supply unlimited quantities of semen to owners of registered Jersey cows within a specified area. In special cases licensees may supply limited quantities of semen to cow owners outside such an area.

Some 70 licences under these arrangements are in operation, including 18 under the Jersey scheme referred to above.

Future Programme.—No spectacular developments are forecast. The majority of new centres to be opened in the near future will be operated by the Milk Marketing Board, who have plans in hand for the establishment, under licence, of centres or sub-centres in Cheshire, Devon, Dorset, Kent, Yorkshire and Montgomeryshire. The Board also contemplate setting up additional centres next year.

Work is in progress in connection with a sub-centre of Dartington Hall at Liskeard, Cornwall, and Messrs. Horlicks Farms and Dairies Ltd. plan to open at least one other sub-centre in Somerset in the near future. A centre in Hertfordshire is also contemplated.

Subject to unforeseeable delays caused by labour shortage and building difficulties, it is probable that the undermentioned centres will be licensed this year:

MAIN CENTRES

Frant Whiligh, Kent
Sturminster, Newton, Dorset
Torrington, Devon
Welshpool, Montgomeryshire
} Milk Marketing Board

SUB-CENTRES

Liskeard, Cornwall (Dartington Hall Centre)
North Somerset (for Ilminster)—Messrs. Horlicks Farms and Dairies, Ltd.
Stokesley (for Shincliffe)—Milk Marketing Board

Most of the development which has so far taken place has been in the provision of facilities for artificial insemination with semen from good quality dairy bulls of appropriate breeds, but it is to be noted that bulls of beef type are available at the Kirton centre. It is not expected that breeders of beef cattle will tend to make use of artificial insemination centres in their type of livestock husbandry to the same degree as will the milk producer, but none the less it will be a matter of policy, in guiding the development of a national service, to ensure that good beef bulls are provided wherever the need exists and the demand is sufficiently great to justify economically the maintenance of bulls of a suitable breed.

Research Work.—Much more research is needed on the subject of artificial insemination. As already mentioned, difficulties relating to the transport of semen are under investigation. It is anticipated that these enquiries will not only facilitate the operation of centres in this country but will throw some light on the practicability of importing and exporting semen successfully. For purposes of control, the Minister has made the Importation and Exportation of Semen and Imported Semen (England and Wales) Regulations, 1946 and, for the time being, the issue of licences under these Regulations is limited to samples being despatched or received for approved research and experimental purposes.

The dilution of semen is another matter calling for further study. The object of this operation is to enable more cows to be served per ejaculation. Further experiments have been planned to supplement the information on this aspect already available from work in this country and abroad.

In conclusion it must be emphasized that whilst artificial insemination as a method of breeding cattle has considerable prospects as

a means of assisting the more rapid improvement of British livestock, due caution must be observed in its development. It will continue to be necessary to breed good bulls for use in the usual way.

Artificial Insemination Centres

Centre (*Sub-centres are shown in italics*)	Operations Started	Breed of Bulls Kept
(a) MINISTRY'S RESEARCH CENTRES		
Reading Shinfield, Reading, Berks	February, 1943 (taken over by Ministry, October, 1945)	British Friesian Guernsey Shorthorn
Guildford 32, High Street, Guildford, Surrey	November, 1943 (taken over by Ministry, October, 1945)	
Bloxham Banbury, Oxfordshire	October, 1946	
Buckland Bampton Road, Buckland, near Faringdon, Berks	October, 1946	
Ruthin Llysfasi Farm Institute, Ruthin, Denbighshire	(1) November, 1943 (Disease control) (2) December, 1945 (Artificial insemination service)	Shorthorn
(b) MILK MARKETING BOARD		
Beccles Walnut Tree Farm, Benacre, Beccles, Suffolk	December, 1943 (taken over by M.M.B., November, 1944)	British Friesian Canadian Holstein Red Poll Shorthorn
Otley Hall Road, Otley, Suffolk	December, 1945	
Carmarthen Johnstown, Carmarthen	October, 1946	Canadian Holstein British Friesian Shorthorn
Cheswardine Cheswardine Hall, Cheswardine, Market Drayton, Salop	January, 1946	Ayrshire British Friesian Shorthorn
Ellesmere	October, 1946	
Little Horwood Bletchley, Bucks	July, 1946	Jersey Shorthorn
Aylesbury Bourbon Street, Aylesbury, Bucks	September, 1945	
Moulton Northants Farm Institute, Moulton, near Northampton	October, 1946	
Shincliffe Shincliffe, near Durham	February, 1946	Ayrshire British Friesian Shorthorn

(c) OTHER INDEPENDENT CENTRES	Operators		
Bromsgrove Sugarbrook, Stoke Pound, Bromsgrove, Worcs	Mid-Worcestershire A.I. Centre, Ltd., Avoncroft College, Bromsgrove, Worcs	December, 1944	Ayrshire Shorthorn
Cambridge The Gravel Pits, Huntingdon Road, Cambridge	Cambridge and District Cattle Breeders' Soc., The Gravel Pits, Huntingdon Road, Cambridge	November, 1943	British Friesian Shorthorn
Dartington Hall Yarner, Dartington, Totnes, Devon	Dartington Hall, Artificial Insemination Centre, Wingett, Dartington, Totnes	January, 1944	South Devon
Ilminster Hort Bridge, Ilminster, Somerset	Messrs. Horlicks Farms and Dairies, Ltd., Slough, Bucks	November, 1944	British Friesian Guernsey Shorthorn
Castle Cary Higher Flax Mills, Castle Cary, Somerset	Ditto	July, 1946	
Wells The Hollies, Wells, Somerset	Ditto	July, 1946	
Kirton Bamber's Yard, Frampton, Boston, Lincs	South Lincs Cattle Breeders' Society, Agricultural Institute, Kirton, Boston, Lincs	November, 1946	British Friesian Shorthorn (Lincoln Red Beef type)
Lyndhurst Grand Hunting Stables, Lyndhurst, Hants	Hampshire Cattle Breeders' Society, GrandHuntingStables, Lyndhurst	February, 1945	British Friesian Guernsey Jersey Shorthorn
Andover "Croham Hurst," Pickett Piece, Andover, Hants	Ditto	January, 1946	
Petersfield "Heathlands," Lavant Street, Petersfield	Ditto	September, 1946	

preparations *after* parturition was useless, as we were able to observe in two mares with agalactia.

These experiments on mares confirm results (unpublished) that we have obtained on other domestic animals: sows, cows and sheep, when we were also able to observe that stilbene influences the development of the udder and the following lactation favourably, when applied locally shortly before parturition, but not if administered subsequently.

CLINICAL COMMUNICATION

Hormonal Treatment of Agalactia in the Mare

by

Professor PEETERS, Professor VANDEPLASSCHE and Professor MASSART

(Veterinary School of the State University, Ghent, Belgium)

Most cases of agalactia in the mare coincide with insufficient development of the udder. The increase in size of a mare's udder occurs especially during the last weeks of gestation. Agalactia of the mare is a very prejudicial phenomenon, which often causes the death of the colt, as is well known by horse-breeders. Especially with race-horses is it a fatal phenomenon, for the subsequent racing career of the full grown animal depends entirely on the growth of the young colt.

As agalactia of the mare is a phenomenon which generally relapses and reappears every year, we tried to induce in six mares, which we knew gave little or no milk at the previous parturition, sufficient development of the udder, by administrating stilbene preparations. We made use of Diethylstilboestrol ointment* in a one per cent. concentration, this preparation being kindly supplied by the U.C.B. firm in Brussels. From 2 to 3 grammes of this ointment was smeared on the udder of the mares every day during the fortnight before the probable date of parturition.

CASES:

I. A six-year-old Belgium Draught Horse (B.D.H.) mare, foaled in 1944. Udder dormant and yielding too little milk. Colt died, 1945: treated during the fortnight preceding the end of pregnancy. Once a day the udder was smeared with 2 to 3 grammes D.E.S. ointment 1 per cent. Foaled five days over time. Udder strongly responsive and milk abundant. The colt died of polyarthritis four days after birth.

II. A B.D.H. mare, foaled in 1943 and 1944, but each time the colt died for lack of milk. 1945: treated in the aforementioned way ten days before the probable date of parturition; foaled 14 days over time. The udder had developed very well and milk was plentiful. Colt grew up normally.

III. A B.D.H. mare, lost her colts in 1943 and 1944 for lack of milk. 1945: treated one week before the probable date of parturition. Foaled six days over time. Udder normally developed. Sufficient milk. Colt grew up normally.

IV. A B.D.H. mare. Her colt died in 1945 for lack of milk. Udder dormant. In 1946: treated four days before probable date of parturition. Foaled 22 days over time. Udder grew abnormally. Foaled normally and had much milk. Colt nevertheless died of polyarthritis after three days.

V. A B.D.H. mare. Her colt died in 1945 for lack of milk; insufficient development of the udder. 1946: treated a fortnight before probable parturition. Foaled eight days over time. Udder responded strongly and much milk formed. Colt grew up normally.

VI. A B.D.H. mare. In 1945 the colt died for lack of milk, Udder insufficiently active. 1946: mare treated ten days prior to probable parturition. Foaled 21 days over time. Udder reasonably developed and plenty of milk. Colt grew up normally.

Discussion

With our stilbene therapy we obtained, in six cases, a favourable result with mares that, at their previous parturition, showed symptoms of agalactia. Without our treatment the secretion of milk would again have been insufficient. In our opinion the explanation of these favourable results is as follows:

(1) Stilbene, locally administered in small continuous doses towards the termination of pregnancy, causes growth and development of the milk gland. This period is selected because it is precisely during the last weeks of pregnancy that the mammary gland exhibits the strongest physiological reaction.

(2) Stilbene, in small doses, also stimulates the function of the anterior lobe of the pituitary and causes a strong secretion of the hormones which are necessary for the development of the gland and the secretion of the milk (prolactin and the glucotropin).

The therapeutic agent used is completely harmless for the mare and her progeny. The birth was not hastened through this relatively small dose of stilbene, which is slowly resorbed and probably quickly disintegrated in the liver and excreted by the kidney. In fact, in all the mares the parturition was delayed, causing some concern to the owner despite the fact that treatment was kept up till parturition. Treatment of the mammary gland with stilbene

* *Formula*: Diethylstilboestrol 10 mg. ; excipiens *q.s.* pro. g. Un.

(*Concluded at foot of previous column.*)

ABSTRACTS

[Single Injection Specific Treatment for Foot-rot in Cattle. Forman, C. R. (1946.) *J. Amer. vet. med. Ass.* **109**. 126-127.]

The author, who is the owner of a large herd of Jersey and Guernsey cattle in a district where the incidence of foot-rot is high, claims that the intravenous injection of 60 grammes of sodium sulphapyridine in 500 c.c. of distilled water is a specific treatment and non-toxic for all forms of the disease. One injection is said to suffice. In larger breeds it may be necessary to increase the dose. No local treatment is advised and the affected animals are left in the herd. All stages of the condition have been treated with equal success, swellings and lameness improving within 24 hours and complete recovery taking place in from two days to two weeks. If articular surfaces are damaged, lameness will probably remain after the infection has been destroyed. Eighty cases are claimed to have been treated successfully. In another group of cases 59 out of 63 cases required only one injection, whilst the remaining four required a second injection four to five days after the first. A dose of 90 grammes of sodium sulphapyridine in 900 c.c. of water has been tried in some cases, and apart from some slight nervous reaction immediately after injection the animals showed no apparent ill effects, but the author believes that this amount is in the neighbourhood of the toxic dose.

J. C. G.

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[The Effect of the Pre-partum Diet of the Cow on the Vitamin A Reserves of her Newborn Offspring. Wise, G. H., Caldwell, M. J., & Hughes, J. S. (1946.) *Science*. **103**. 616.]

During the immediate pre-partum period healthy Ayrshire, Holstein and Jersey cows were fed a basal ration of good-quality alfalfa hay, sorghum silage and a concentrate mixture. They were divided into three groups, the first being restricted to this ration, the second being grazed on pasture for from 14 to 90 days in addition, and the third receiving a "dry vitamin A" supplement for from eight to 45 days, allowing one million U.S.P. units of vitamin A per day. Samples of venous blood were drawn from the calves, usually within four hours after birth and always before colostrum ingestion.

There were no marked differences between the vitamin A reserves of calves from cows in groups one and two but there was significantly higher storage in those from cows in group three. The vitamin concentration in the serum of these calves approximated that of other colostrum-fed calves when twelve hours of age. The vitamin A concentration in their livers was considerably greater than in the average calf twelve hours after receiving colostrum, but much less than one fed colostrum for four days. The reason may be that the esterified form of vitamin A, present in "dry vitamin A," traverses the placenta more readily than the alcohol form which is the principal type in the bovine circulation. It is also possible that the ester form is more resistant to destructive processes in the placenta, and to metabolic activity in the foetus.

There appeared to be no advantage in giving the vitamin A supplement before the last two weeks of gestation. The low values of vitamin A normally found in the foetal calf liver are inadequate for post-natal health, and the vitamin A potency of the first supply, the colostrum, also depends on the pre-partum diet of the dam. Thus a high initial vitamin A reserve in a newborn calf should have practical value in the maintenance of its post-natal health.

J. O. L. K.

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[Studies on Brucellosis of Swine. II.—Exposure and Re-exposure Experiments with *Brucella suis*. Hutchings, L. M., Delez, A. L., & Donham, C. R. (1946.) *Amer. J. vet. Res.* **7**. 11-20 (6 refs.)]

The authors inoculated intravenously or subcutaneously with *Br. suis* 19 pigs previously immunized with viable culture and compared the results with those obtained with 15 similarly inoculated pigs, not previously immunized. In both groups, pigs became infected. There was evidence of a small residual immunity in the immunized group but, as abortions were not produced in either group, the results were not decisive. Of six normal gilts exposed to the same infection at breeding time, one definitely aborted and two others may have aborted. Six sows, exposed to infection during their first pregnancy, were again exposed by feeding aborted material after their second breeding. Five of these showed some evidence of re-infection as compared with all five controls, two of which aborted. The evidence suggested that swine previously exposed to *Br. suis* are somewhat more resistant to the organism than normal animals of the same age but not sufficiently so to enable them to withstand re-infection.

A. W. G.

QUESTIONS IN PARLIAMENT

Feeding-stuffs

Mr. Hurd asked the Minister of Agriculture when he expects to be able to provide more feeding-stuffs for chick rearing in order that poultry farmers can by timely restoration of laying flocks use to advantage the large supplies of grain that will be shipped to this country in 1947 and so save the high cost of dried eggs imported from the U.S.A.

Mr. De la Bere asked the Minister of Agriculture whether, in connection with the additional cattle feeding-stuffs purchased by the Ministry of Food, he will, in conjunction with the Minister of Food, issue a short statement regarding the additional supplies which may be available for 1947 to farmers, stockbreeders and poultry keepers.

Mr. T. Williams: The Government is making constant efforts to buy more feeding-stuffs in overseas countries and as soon as larger supplies can be obtained and shipped to this country, ration scales for all classes of stock will be reviewed. Certain increases in rations including chick rearing rations during the period January-April next, were announced on December 11th. These increases are as great as are warranted by the supplies available. Our prospective supplies are, as yet, too uncertain to justify larger increases or to enable any statement to be made in regard to the likely levels in 1947 of rations for poultry or any other class of stock.

Mr. Hurd: Does the Minister hold out any hope of being able to give a definite lead to the farmers by the middle of January when his colleague, the Minister of Food, as he has told us, hopes to know how he stands for cereals?

Mr. Williams: That would be attempting to enter into the realm of prophesy with all the known difficulties regarding shipping, transport and all the rest of it in any of the exporting countries.

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Poultry Breeding Stock (Export Licences)

Sir John Barlow asked the Minister of Agriculture whether the restrictions that have hitherto been imposed on the export of poultry breeding stock, day-old chicks and hatching eggs to Europe and other countries will be relaxed or removed as a means of re-establishing pre-war connections with such countries and to assist breeders at a time when the market for breeding stock is limited in this country owing to the shortage of feeding-stuffs for commercial and domestic poultry keepers.

Mr. T. Williams: The question of the export of livestock from this country to the Continent of Europe has recently been reviewed, and it has been decided that applications for export licences can be more freely granted in respect of poultry breeding stock, young chicks and hatching eggs, and also of pedigree cattle, sheep and pigs. Exports will not be allowed to an extent which would jeopardize the re-establishment of our own flocks and herds and the future home production of milk, meat and eggs.

Sir J. Barlow: Is the Minister aware that this news in respect of poultry will be gratefully received by accredited poultry dealers in particular, and will he give his reply the greatest publicity?

Mr. Williams: We will do our best.

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Farm Institutes

Mr. Dye asked the Minister of Agriculture how many proposals for the establishment of new farm institutes have received his approval during 1946; and what progress has been made towards instituting a farm institute in every county in England.

Mr. T. Williams: As a report on farm institutes from the Joint Advisory Committee on Agricultural Education making recommendations for future development, is expected by the end of the year, I have restricted my approval of proposals submitted by individual authorities during the year to those, numbering eight in all, in which places may be required to meet short-term ex-Service training needs. There are 18 farm institutes in England and Wales and proposals for an additional eleven have been authorized this year and last on the basis indicated. The extent to which the general long-term objective to which my hon. Friend refers is attainable during the next few years is a question on which I am awaiting the guidance of the Joint Advisory Committee.

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Dogs, M.E.F. (Destruction)

Sir G. Fox asked the Secretary of State for Air whether he is aware that an order was made on November 27th, at the M.E.F. station where No. 32 squadron is serving that all dogs thereon were to be shot with the exception of five registered dogs; that the weapon used for their destruction was a 12-bore sports gun using six shot cartridges; that one of the dogs which was tied to another for

destruction was blinded in one eye by shooting, but is still living at the station; and whether steps will be taken to prevent unnecessary cruelty of this kind.

Mr. P. Noel-Baker: Three dogs at this station were suspected of rabies and their destruction was ordered. Two were destroyed but with the third the cartridge misfired, and the dog escaped to its owner; its eyes were not affected, nor was it tied to another dog.

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The Agriculture Bill

Before Parliament adjourned for the Christmas Recess the Government formally introduced an Agriculture Bill to give effect to their policy for agriculture, which is based on a system of guaranteed prices and assured markets for the principal products of the farm.

The Bill also provides for the dispossession of farmers and landowners who persistently fail "in the responsibilities attached to the occupation and ownership of land." It proposes the establishment of a commission to manage and develop land which the Government may acquire by voluntary negotiation, or compulsorily in cases of dispossession, or where public ownership is held to be the only means of securing its full productive use. County committees similar to the war agricultural executive committees will be established on a permanent basis. The Council of Agriculture of England and that of Wales, also the Agricultural Advisory Committee, will be abolished. The immediate reason for this step is the fact that the constituent bodies that elected the bulk of the two Councils are to be abolished and the agricultural duties of the County Councils are to be reduced, so that the establishment of a Council composed mainly of representatives of County Councils ceases to have validity. County Councils will, in fact, be relieved of the statutory responsibility of appointing their own agricultural committees, some of whose more important functions will be permanently transferred to the County Agricultural Executive Committees.

ROYAL COLLEGE OF VETERINARY SURGEONS

Membership Examinations, December, 1946

List of Successful Candidates

(*Continued from page* 615)

Glasgow

First Year:

Bunyan, F. S. R.
de Boni, Miss R.
Dexter, M. R.
Gallagher, Peter
Hugh, A. R.
McKerrell, A. L. L.
Ricketts, Miss B. C.
Robertson, W. W. (B)
Russell, D. A.
Sachs, Max
Thomson, Thomas
Thorburn, H.
Wilcox, W. H.
Young, D. K. M.

(B) denotes credit in Biology.

Second Year:

Aitken, George
Anderson, R. Y.
Beresford-Jones, W. P.
Duncan, George
Ferguson, Thomas
Gibson, A. C.
Gilchrist, J. A. S. (P)
Gittleson, M. L.
Glas, Michael
Gross, Miss I. M.
Hall, F. P.
Harkness, A. C.
Horn, W. R. (H)
Irvine, G. B.
Lindsay, T. P.
MacLennan, Donald
Orr, R. S. (H)
Roger, S. A.
Strang, A. L. D
Swift, A. G.
White, Miss C. H.
Wilson, A. G.
Wilson, Miss J. S. S.

(P) denotes credit in Physiology, etc.
(H) denotes credit in Histology, etc.

Third Year:

Dunlop, J. C.
Finklestein, B. (A)
Greig, Miss Jean
Glenroy, J. M.
Grisedale, J. A.
Jackson, W. T.
Lustig, Martin
Mackay, Angus
*Mair, C. P.
Ritchie, N. D.
Whyte, Miss E. N.

* denotes 2nd class honours.
(A) denotes credit in Anatomy.

Fourth Year:

Anderson, J. A.
Bryson, William
Campbell, A. D.
Crawford, Miss D. H.
Crawford, Miss E. T
Garven, James
MacInnes, T.
Murphy, Miss L. I.
Nimmo, I. S.
Williams, Miss L.

Final Year:

Campbell, R. S. F.
Dunn, A. M.
Lamond, J. L.
Locke, Hugh
Macdonald, D. K.
McGown, S. M.
Murdoch, W. A.
Smith, Miss A. M.

NOTES AND NEWS

Diary of Events

Jan. 2nd.—Meeting of the Central Veterinary Society at the Conway Hall, W.C.1, 2 p.m.

Jan. 9th.—N.V.M.A. Committee Meetings at 36, Gordon Square, W.C.1: General Purposes and Finance (morning), Veterinary State Medicine (afternoon).

Jan. 10th.—N.V.M.A. Technical Development Committee at 10 a.m., Council Meeting at 11 a.m. (Both at the Connaught Rooms, Great Queen Street, Kingsway, London, W.C.2)

Jan. 10th.—Meeting of the Association of Veterinary Teachers and Research Workers (Founder Members resident in Southern Region), at the Royal Veterinary College, N.W.1, 2.15 p.m.

Jan. 15th and 16th—R.C.V.S. Committee Meetings.

Jan. 17th.—R.C.V.S. Quarterly Meeting of Council.

July 28th—Aug. 1st.—N.V.M.A. Annual Congress in Edinburgh.

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N.V.M.A. ANNUAL CONGRESS, 1947.

It has been decided to hold the 1947 Congress in Edinburgh from July 28th to August 1st inclusive. It is desirable that applications for accommodation should be made as early as possible as hotels and boarding-houses are already booking for next summer.

If any members fail to obtain suitable accommodation they should get in touch with the Hon. Local Secretary, Professor A. Robertson, Royal (Dick) Veterinary College, Edinburgh, 9, who will endeavour to assist them.

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New Appointments to the Staff of the Ministry of Agriculture

As notified in recent issues, Council, N.V.M.A., has decided that no advertisements for appointments to the veterinary staff of the Animal Health Division of the Ministry of Agriculture shall be accepted for *The Veterinary Record* pending the settlement, in a manner satisfactory to the Association, of the question of the status and salaries of the Veterinary Officers of the Division.

In the meantime, any members of the profession who may, nevertheless, contemplate applying for such posts, should these be advertised elsewhere, are requested first to communicate with the General Secretary of the Association at 36, Gordon Square, London, W.C.1.

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R.C.V.S. OBITUARY

Summers, William, Allendale, Northumberland. Graduated N. Edinburgh, July 22nd, 1880. Died December 10th, 1946; aged 89 years.

Mr. William Summers, M.R.C.V.S.

Mr. William Summers, whose death we record with much regret, was a Dale farmer and veterinary surgeon with an extensive practice in Northumberland, Cumberland and Durham. The *Hexham Courier*, in a tribute, says of him: "One of the best-known and popular personalities of the Dale, Mr. Summers was owner-occupier of Milescott for over 60 years. A staunch supporter of the Allendale Agricultural Society, he was the oldest member of the management committee. In his younger days he was a keen

follower of the hounds, taking part in the long hunts over the moors of Hexhamshire, Allenheads and Alston. Mr. Summers was a great church worker, and when he resigned two years ago he had been sidesman at St. Mark's Church, Ninebanks, for 39 years."

The funeral at Ninebanks followed service in St. Mark's Church, conducted by the Vicar, the Rev. Stanley White. The large attendance included Mr. F. Pickering, M.R.C.V.S., of Hexham, Mr. D. M. Cochrane, M.R.C.V.S. (representing the Ministry of Agriculture and Fisheries), and Mr. T. H. Hudspith (representing the Allendale Agricultural Society).

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CO-ORDINATION BETWEEN FARMERS AND VETERINARIANS

With the object of increasing the co-ordination between the practical farmer and the veterinary profession, a conference was held in Ayr attended by representatives of the Association of Certified and T.T. Milk Producers and members of the veterinary profession practising in various dairying districts in Scotland.

In opening the conference, the Chairman, Mr. A. W. Montgomerie, Dunure Mains, Ayr, declared that the Association's efforts to co-ordinate the work of those engaged in research into dairy cattle diseases with the work of the practical farmer had proved fruitful. He recognized that it was dangerous to draw any general conclusions from any one or two good results obtained in the field, but farmers heard so many conflicting reports regarding, for example, cures for mastitis, that it was obviously advisable to get the facts from those actively engaged in veterinary practice.

In the discussion which followed, the experiences in various parts of the country of penicillin treatment for mastitis were fully discussed. The general opinion was that, while the results so far achieved gave good grounds for hope that a solution would ultimately be found, the experience of penicillin was of too short duration to enable anyone to arrive at any definite conclusion.

Several speakers expressed the view that powers for a much greater supervision of milk production at all its stages should be vested in the veterinary profession in whom the farmers had confidence. It was decided that further efforts should be made to encourage the work which this conference had begun.

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PARASITES OF HILL SHEEP

Problems connected with parasites of hill sheep were discussed at a conference held in Edinburgh University's Department of Zoology, King's Buildings, West Mains Road, on Saturday, under the auspices of the Hill Farm Research Committee of the Scottish Agricultural Advisory Council, reports *The Scotsman* in its issue of December 16th.

Professor James Ritchie, of Edinburgh University, who presided at the opening session, said that our agricultural research institutes were performing a service of almost incalculable value to stock-breeders and the country. To take but one instance, the value to the agricultural community of the work done at the Moredun Research Institute of the Animal Diseases Research Association was moderately expressed at £300,000 a year. It was a relatively small institute, which was very cheaply run, and it was saving the farming community that large sum as a result of its discoveries in sheep diseases and by its preparation of preventive vaccines.

Dr. J. Russell Greig, C.B.E., of the Animal Diseases Research Association, who was chairman of the afternoon session, said there now existed a great mass of established knowledge which could be applied by the farmer in the control of worm parasites, but one was rather appalled at the lack of the application of that established knowledge.

Dr. I. W. Parnell, of the Department of Zoology of Edinburgh University, estimated the number of sheep which suffered from helminthiasis at over half a million in a year. Unfortunately it was a loss which farmers often did not realize. Even when worms killed sheep they seldom did so in large numbers in a short period, so that it was not brought home to the farmer so forcibly. The most serious losses they caused were in lack of milk for their lambs and the lowering of wool and mutton production. Much work must be done to determine the ideal dosing programme for different districts, since dosing appeared to be the only method of control which was immediately applicable.

There was no doubt that tapeworms were exceedingly common in sheep on hill farms in Scotland, said Dr. Rayski, of Edinburgh University Zoological Department, but so far as he was aware there were no attempts to control these worms by treatment. That was probably due to prevalent ideas that tapeworms were of little importance and therefore did not justify the expenditure of time and money on dosing. In his view heavy infestations had a very important influence on growth and condition.

Dr. D. O. Morgan, Edinburgh University, said that worms in sheep, and indeed in grazing animals generally, were picked up from the grass in the form of larvae which were free or inside intermediate hosts. It was, however, very fortunate that worms, unlike bacteria, did not multiply inside the host, and each worm that developed in a sheep had to be picked up separately from the grass. Even so, such was the capacity of worms for egg-production that a heavily stocked pasture might become dangerous to the animals in a matter of weeks.

Mr. J. Allan Campbell, B.A., M.R.C.V.S., of the Department of Agricultural and Forest Zoology, Edinburgh University, dealing with biological investigations on the ecology of the sheep-tick, said there was no stage in the life history where there was a swarming or congregation of individuals except where they were feeding on a host. Consequently, chemical treatment of domestic stock was the only positive method of attack, and the ultimate control of the pest probably depended on improved dip technology.

Mr. G. B. S. Heath, B.SC., M.R.C.V.S., Ministry of Agriculture and Fisheries, Veterinary Laboratory, Weybridge, said that a new method of assessing the values of anti-tick dips had been evolved. D.D.T. had been the main acaricide used during recent years, and one dip containing D.D.T. was capable of preventing any female tick from engorging during the three to four weeks following dipping. Some evidence existed to show that females which engorged on D.D.T. dipped sheep did not survive to lay eggs.

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CENTENARY OF THE SMITHSONIAN INSTITUTION

The greatest of American scientific organizations, the Smithsonian Institution of Washington, which stands very l igh not only in the opinion of America but of the civilized world, is this year celebrating its centenary. In honour of the occasion an attractive illustrated volume outlining its first 100 years of work has been issued.

It perhaps reflects credit on both nations (says *The Times*) that this famous American establishment was founded by an Englishman, James Smithson (1765-1829), who never visited America. An illegitimate son of the first Duke of Northumberland, he became a Fellow of the Royal Society in 1787 because of his proficiency as a chemist and mineralogist. He lived much of his later life in France and died in Italy. By his will he left his fortune of about £120,000 first to a nephew and on the latter's death, failing an heir, "to the United States of America, to found at Washington, under the name of the Smithsonian Institution, an establishment for the increase and diffusion of knowledge among men."

Because of various formalities it was not until 1846 that the Smithsonian Institution was launched. Since then it has covered a large field of knowledge, including natural history, anthropology (particularly the study of the Red Indian), astronomy, engineering, and art. It has sponsored many voyages of exploration, and its establishment includes not only the National Museum but a zoological garden, an astrophysical observatory with branches in Chile, California and New Mexico, and—not least—the National Gallery of Art, where the late Andrew Mellon's magnificent collection of paintings is housed. Its publications are known throughout the world.

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ADDRESSES OF DISEASE-INFECTED PREMISES

The list given below indicates, first, the country in which are situated the premises on which disease has been confirmed, followed by the postal address and date of outbreak.

FOOT-AND-MOUTH DISEASE:

Dorset.—Barton Farm, Sherborne, Dorset (Dec. 19th); Sherborne Abattoir, Sherborne, Dorset (Dec. 20th).

Middlesex.—Cripps Farm, Harefield, Middlesex (Dec. 19th).

Somersetshire.—Lower Farm, Chilton Cantelo, Yeovil, Somerset (Dec. 19th).

SWINE FEVER:

Lincs. (*Lindsey*).—Home Farm, Faldingworth, Market Rasen, Lincs. (Dec. 18th).

SHEEP SCAB:

Notts.—Greenlands, Leverton Road, Retford, Notts. (Dec. 19th).

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ADVERTISER'S ANNOUNCEMENT

Burroughs Wellcome & Co. announce the adoption of a five-day week for their London offices and warehouse, commencing on January 1st, 1947. Until further notice, an emergency staff will be in attendance on Saturday mornings at 12, Red Lion Square, W.C.1. Tel.: CHAncery 6621, from 9 a.m. to 12.30 p.m., to deal with urgent orders.